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RESEARCH PAPER

Dynamics of growth and phytomass allocation in seedlings of *Pistacia atlantica* desf. versus *Pistacia vera* L. under salt stress

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Abstract

Salinity represents a major environmental constraint which can severely limit plant establishment in the arid and semi-arid areas in Tunisia. The present work was carried out to study the response of *Pistacia vera* L. and *Pistacia atlantica* Desf. seedlings to salt stress. Evaluation of salinity effects on both species was investigated using five increasing NaCl concentrations (0, 20, 40, 60 and 80 mM) during 60 days. The exposure to NaCl at seedlings stages, affects the majority of the studied parameters. Morphological parameters, such as height of shoot, number of green leaves, leaf area and consequently, phytomass allocation were significantly decreased. Additionally, *P. vera* accelerated leaf senescence. Both species showed a preferential allocation of the resources in favor of the roots for concentrations of NaCl lower than 40 mM. Whereas, only *P. atlantica* maintained a root growth for higher concentrations. Under salt stress, the specific leaf area decreased in *P. atlantica* ($p<0.001$) and translate a mechanism of tolerance to salinity. It appeared that both species tolerate concentrations lower than 40 mM of NaCl. However, for higher concentrations, *P. atlantica* seedlings are more tolerant.

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Introduction

The problem of salinity becomes more and more extensive in the majority of the countries in the process of development, mainly due to water scarcity (Rengasamy, 2010). Approximately 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Zhu, 2001; Yamaguchi and Blumwald, 2005; Sun *et al.,* 2009; Wu *et al.,* 2010). In Tunisia, the saline lands are relatively frequent, occupying a surface of 1.5 million hectares, about 25% of the total cultural soils of the country (Jbira *et al.,* 2001; Ben Ahmed *et al.,* 2008). Salt stress affects plant growth through a double osmotic and ionic constraint followed by nutriment imbalance. Firstly, high concentrations of salts in the soil solution impose an osmotic stress on cell water relations which leads to reduction in water availability to plants and cellular dehydration (Sairam *et al.,* 2002; Chartzoulakis, 2005). Secondly, an ionic toxicity caused by ions accumulation (mainly Na^+ and Cl^-) at high levels (Tavakkoli *et al.,* 2011). Besides, nutriment deficiencies occurred due to the interaction of these ions with other nutritive elements (Tejera *et al.,* 2006; Rejili, 2007; Evelin *et al.,* 2009). The disturbance of potassium (K^+) nutrition resulting from potassium–sodium interaction is often reported (Cramer *et al.,* 1987).The salinity of soil and water of irrigation counts among the principal factors which limit plant growth and productivity (Zahran, 1999; Flowers, 2004; Parida and Das, 2005). Species develop morphological and physiological changes in the dynamics of growth, such as leaf number, plant height and leaf area reductions, increases in root growth and changes in dry phytomass allocation, as increases in leaf senescence, a decrease in specific leaf area and greater biomass allocation to roots. Plant growth reduction is often accompanied by inducing the senescence of leaves, mechanism adopted by the pistachio tree and other Mediterranean species (Colla *et al.,* 2006).The leaf expansion decreases immediately following an increase in the salt concentration and the duration of the treatment (Munns, 2002; Bartels and Sunkar, 2005). The seedlings of pistachio tree reduce their growth when they are subjected to high salinity (Chelli-Chaabouni *et al.,* 2010). In spite of the direct exposure of the root to salinity, its growth is less affected by salt than the shoot (Parida and Das, 2005). It was reported that species surviving under optimal conditions present high specific leaf area (SLA), high relative growth rate (RGR) and high allocation of biomass to shoots, while species with low SLA and low RGR, allocate more biomass to roots in unfavourable habitat (Poorter and Garnier, 1999). The early stage of seedling growth is a critical phase in arid and semi-arid lands where plants are exposed to a series of abiotic and biotic stress. Plants exposed to salt stress are negatively affected with reduction in establishment and growth.

Pistacia vera and *Pistacia atlantica* are two pistachio species, cultivated and spontaneous respectively, belonging to the Anacardiaceae family. They are species of economic, ecological and medicinal interests (Sari *et al., 2010*; Tomaino *et al.,* 2010). They are characteristic of the arid and semi-arid area. In Tunisia, according to Chaieb and Boukhris (1998), they are cultivated on all the territory, with prevalence in the arid and semi-arid areas. *P. vera,* which produces edible fruits, can survive in areas where annual rainfall is lower than 200 mm (Chaieb and Boukhris, 1998). This species supports the dryness better than the olive, the almond and the fig tree, considered as a typical xerophytes tree, with the advantage to resist to salinity (Behboudian *et al., 1986*; Rieger, 1995) and to tolerate extreme temperature, until -30°C in winters and 50°C during the summer. But for others, it is classified as a medium tolerant to salinity (Ebert, 2000).

P. atlantica is a very powerful woody and spontaneous species, adapted to the constraining pedo-climatic conditions of the arid and semi-arid areas. However, it is observed only on the degraded forests as aged and dispersed individuals, not exceeding 1500 individuals in Tunisia (Ghorbel *et al.,* 1998), being threatened with extinction. Their rusticities in front of edaphic and climatic constraining factors and their low natural regeneration rates made very interesting to study these species. The reintroduction of tolerant species in the degraded lands could limit the extension of the marginal zones (Melgar *et al.,* 2006; Tattini *et al.,* 2009). *P. atlantica* is also useful to receive the graft of fruit pistachio (Gijón *et al.,* 2010), improving vigor and production. However, the mechanism of improvement still not understood. In woody crops, rootstocks are mainly used in order to improve some of the tree characteristics as tree vigor, crop production or production quality (Olien and Lakso, 1986; Cohen and Naor, 2002; Weibel *et al.,* 2003), thus, the graft may influence the movement of substances in the xylem such as ions (Jones, 1971; Tavallali and Rahemi, 2007), water status (Olien and Lakso, 1986) and plant-growth regulating hormones (Kamboj *et al*., 1999). In this context, Atkinson *et al.* (2003) and Solari *et al.* (2006) showed that the tree hydraulic conductance associated with specific rootstocks are determinant of growth potential in grafted trees. The used pistachio rootstocks are *Pistacia vera* L, *Pistacia integerrima* L., *Pistacia terebinthus* L. and *Pistacia atlantica* Desf (Karimi and Kafkas, 2011). The most used rootstock in Tunisia are *P. atlantica* and *P. vera*, but Germana (1997) reported that *P. atlantica* is more susceptible to drought stress than *P. terebinthus*. The present study was aimed to: a) assess comparatively the effect of increasing concentrations of NaCl on the dynamics of growth and phytomass production in *Pistacia vera* and *Pistacia atlantica* at the seedling stage, b) identify the principal physiological mechanisms of salt stress tolerance for the endangered *Pistacia atlantica* versus *Pistacia vera* and c) find a potential relationship between the improvement of *P. vera* vigor since grafted on *P. atlantica* and salinity tolerance.

Materials and methods

Materials and culture conditions

The objective of this experiment is to study the response of *Pistacia vera* and *Pistacia atlantica* to the salinity. The seeds of *Pistacia vera* (Mateur variety) were sampled in the region of Sidi Aïch (Tunisian South-East zone), while the seeds of *P. atlantica* were collected from wild trees in Meknassy (Tunisian West-Central zone) in 2009.

Seedlings production

The production of the seedlings was made in the laboratory at a temperature of 22°C and a photoperiod of 10h/14h light/obscurity. In order to avoid tegumentary inhibition, the seeds of *P. atlantica* underwent a mechanical scarification (Removing the pulp). The external hulls of *P. vera* were also removed. Germination took place in germinators filled with a mixture of peat (2/3) and sand (1/3). Transplantation was carried out after one month of growth. The seedlings at the stage of four to five leaves were then, transplanted in plastic pots, at a rate of an individual per pot. This later is of conical form, 15 cm in diameter and 13 cm in depth, whose capacity is two liters. The contents of the pots were pure sand, really washed, in order to avoid the interference of the trophic factor. The whole pots received the same amount of watering (200 ml) at a rate of two irrigations per week until obtaining vigorous and healthy individuals aged of 70 days.

Treatments applied

Since the sodium chloride (NaCl) represents the major source of salt in the ground and water of irrigation (Turkan and Demiral, 2009), it was used as the source of salt throughout this experimentation. Five increasing concentrations of NaCl were applied: 0, 20, 40, 60 and 80 mM of NaCl, respectively 0, 1.17, 2.34, 3.51 and 4.68 g of salt per liter of nutritive solution, at a frequency of 2 waterings per week. The individuals received a Hoagland solution. From 1st May 2010, the saline treatment was applied during 60 days. The test related to two species, five increasing concentrations of NaCl and 12 repetitions for each one, giving a totally of 120 pots. The culture was placed under a shelter protected by netting at an ambient temperature between 27 and 30°C, a relative humidity of 70% and a photoperiod of 12 h/12 h light/obscurity.

Measurement parameters

During the experiment, several parameters were measured. The green leaf number was estimated once per week by counting the number of green leaves per individual which appear along the experiment (n=6).

The senescent leaves were assessed as soon as the leaf senescence appeared in the treated individuals until the end of the test. A leaf is considered senescent when its reserves are completely impoverished, being thus, dropped from the individual. The Height of shoot was measured using a graduated ruler on an average of six plants for each treatment. This parameter was measured once per week (Expressed in cm). The leaf area by individual was given after the scanning of the leaves by a scanner and analyses of the photographs by the software of image processing Mesurim pro. 8 (Expressed in cm^2 by plant). This parameter was carried out at the end of the test ($n=6$). The shoot and root phytomass were obtained by separating the roots from shoots under water. Then, shoot phytomass (SP, in g per plant) and root phytomass (RP, in g per plant) were determined by weighing after a drying of the vegetable biomasses in a drying oven at 80°C during 48 h. This sampling was carried out at the end of the test ($n=6$). The RP/SP ratio was calculated. It is the relationship between root phytomass (RP) and shoot phytomass (SP). It is given as: $Rate=(RP/SP)*100$. The specific leaf area (SLA) is the relationship between the leaf area of the leaves (LA) and the corresponding dry mass (DL), expressed in cm^2/g ($n=6$). It is given as: $SLA=(LA/DL)$.

Statistical analyses

The results were subjected to the variance analysis (ANOVA) according to a factorial model with fixed factors, by using the software of statistics SPSS (Version 11.5). Then, the results were presented in the shape of the curves, joining average values framed by their standard deviations. The software SigmaPlot (Version 11.0) was used to prepare the various features.

Results

Effect of salt stress on growth of seedlings

In control seedlings, the number of green leaves increased significantly ($p<0.001$). Indeed, the average of the number of leaves increased reaching at the end of the experiment 18 leaves in *P. atlantica* and 13 leaves in *P. vera* (Fig. 1). Following the salt concentrations, probably high on plant, particularly concentration 80 mM, in *P. vera*, not only new leaves didn't appear but also, the formed leaves were desiccated gradually during the experiment. At the end, 56 days of the treatment, the individual was completely senescent (Fig. 1). Under the same conditions of stress, 80 mM also induced an inhibition of the neo-formation of the leaves throughout the test without leaf senescence (Fig. 1). At this level of concentration, the salt stress marked a reduction of only 57%. Thus, the differences between the two species are highly significant ($p<0.001$).

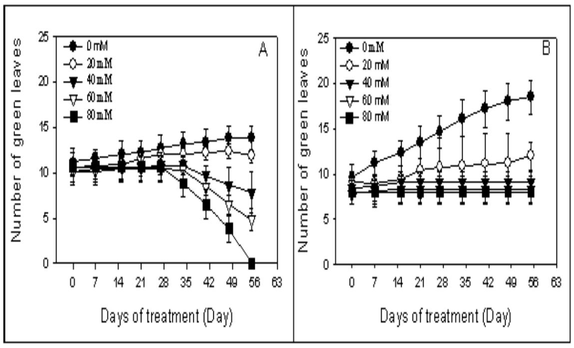

Fig. 1. Salinity effect (0, 20, 40, 60, 80 mM NaCl) on the number of leaves of *Pistacia vera* (A) and *Pistacia atlantica* (B) over a period of 60 days. Each value is the average of 6 replicates. Vertical bars indicate the standard error.

Salt stress marked a highly significant difference between the two species ($p<0.001$) for the leaf senescence. Contrary to *P. atlantica* seedlings, which maintained their leaves alive under salt stress throughout the experiment and even for the highest tested NaCl concentration, the seedlings of *P. vera* triggered after 35 days of the treatment, a premature leaf senescence, largely dependent on the applied NaCl concentration (Fig. 2). Indeed, the number of senescent leaves increased gradually throughout the experiment. The senescent leaves represented 15, 38, 61 and 100% for 20, 40, 60 and 80 mM of NaCl, respectively.

In control seedlings, the differences between the two species are highly significant for the leaf area ($p<0.001$). Indeed, at the end of the test, it reached 12 and 5.2 cm^2 in *P. vera* and *P. atlantica*, respectively (Fig. 3). Thus, the effect of the saline stress on the leaf area is highly significant ($p<0.001$) and the excess of salt in the soil (80 mM) reduced the leaf area ($p<0.001$) by 80 and 89% for *P. atlantica* and *P. vera* seedlings, respectively.

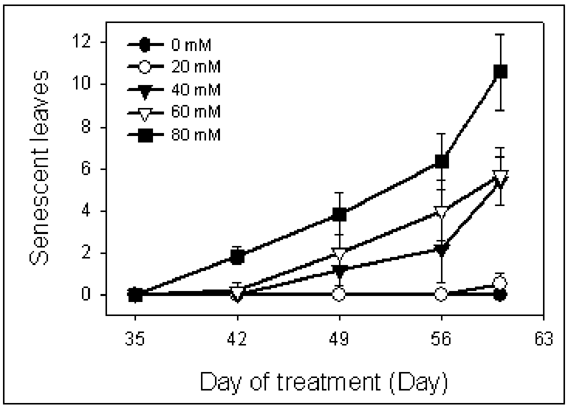

Fig. 2. Salinity effect (0, 20, 40, 60, 80 mM NaCl) on senescent leaves in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

Under salt stress, the specific leaf area showed differences largely depending on the species as well as the tested NaCl concentrations (Fig. 4). Indeed, in *P. atlantica*, the SLA decreased while the salinity increased. The variation of shoot height along the experiment reveals differences between the applied treatments ($p<0.001$) and the species ($p<0.001$) (Fig. 5). In control seedlings, the height of *P. atlantica* moved from 3.90 to 6.85 cm at the end of the test. On the contrary, the height of *P. vera* remained variable between 9.30 and 10.75 cm, whereas, for the seedlings subjected to salt stress concentration 80 mM induced an inhibition of the growth in height for both species. However the height decreased in *P. vera* seedlings against a non significant effect in *P. atlantica* seedlings under 20 and 40 mM of NaCl. At the end of the experiment, the reduction in height reaches 51% in *P. atlantica* against only 17% in *P. vera* for 80 mM.

Effect of salt stress on the phytomass allocation

The analysis of Fig. 6 shows that shoot phytomass was reduced in the seedlings subjected to the various NaCl concentrations as a result of the reduction of the number of green leaves, the height of the individuals, the leaf area of both species and the increase in the number of senescent leaves in *P. vera*. This reduction is marked in *P. vera* for the various concentrations of NaCl and particularly at 80 mM. Whereas in *P. atlantica*, even for high NaCl concentration, the individuals reduced SP by 45%, compared to *P. vera* (88%) that increased leaf senescence.

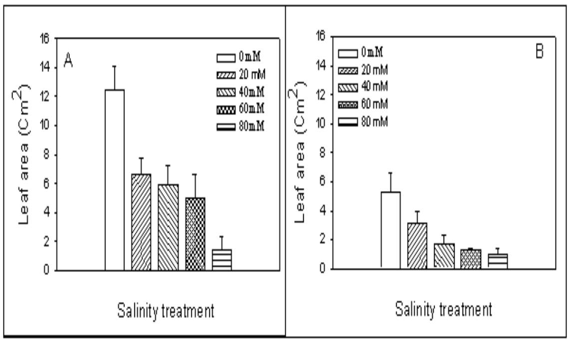

Fig. 3. Leaf area variation in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

Root phytomass (RP) increased for the applied saline treatments (20 and 40 mM) for both species (Fig. 7). However, for higher NaCl concentrations (60 and 80 mM), RP was reduced. This reduction is marked in *P. vera* (87%) at 80 mM, whereas in *P. atlantica*, even for the concentrations of 60 and 80 mM, RP was maintained at higher values compared to the control ones (41%).

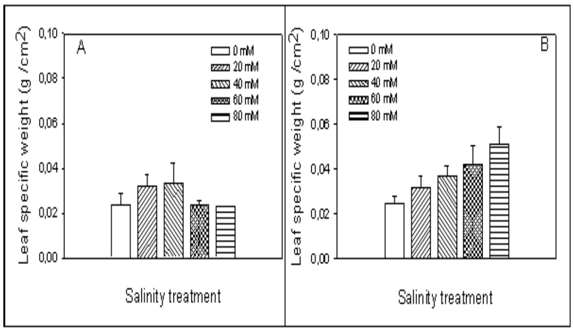

Fig. 4. Specific Leaf Area in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

The RP/SP ratio increased under salt stress, except for 80 mM of NaCl (Fig. 8). Thus, the salinity stimulated the roots growth, especially for *P. atlantica* seedlings ($p<0.001$).

Discussion

Dynamic of growth under salt stress

The NaCl causes a reduction in plant growth (Shannon and Grieve, 1999; Munns, 2002; Parida and Das, 2005; Chelli-Chaabouni *et al.,* 2010; Correia *et al.,* 2010). It is considered as one of the first manifestations of salt stress (Parida and Das, 2005) on morphological and physiological traits. It appears on leaf area, leaf number, mechanism adopted by the pistachio tree and other Mediterranean species (Munns, 2002; Kchaou *et al.,* 2010) to reduce the water losses by transpiration. The reduction in the growth can be explained by the reduction in the photosynthesis and in the leaf area (Parida and Das, 2005). The salt stress causes an immediate reduction in leaf expansion which is inhibited by increasing the salt concentration and duration of treatment (Curtis and Läuchli, 1987; Parida *et al.,* 2003; Abbruzzese *et al.,* 2009), thus, the new leaves develop slowly and the senescence of the old leaves accelerates. This reduction in leaf growth is the result of inhibition of the cellular divisions under abiotic stress, as salt and water stress. Several authors showed that the reduction of the shoots growth in the seedlings of pistachio is related to disturbances of the growth regulators, as the abscissic acid and the cytokinin (Termaat *et al.,* 1985; Kuiper, 1990). In fact, a decrease in the cytokinin hormone was recorded in *Pinus sylvestris*, *Pinus koraiensis* and *Abies hallophylla* (Liu *et al.,* 1998). Also, several studies reported that abscissic acid increased significantly under salinity stress (Mulholland *et al.,* 2003; Zhu *et al.,* 2005; Albacete *et al.,* 2008; Zorb *et al.,* 2013). Moreover, the free auxin decreases according to species (Wang *et al.,* 2001; Ghanem *et al.,* 2008; Salah *et al.,* 2013; Zorb *et al.,* 2013). The reduction of growth can be due also to a nutritional deficiency in stressed plants (Chelli-Chaabouni *et al.,* 2010).

The intensification of salinity is accompanied by reductions in green leaves and their dimension and the stems length, and increases in senescent leaves, thus the morphological parameters are sensitive to the double osmotic and ionic constraints under salt stress (Munns, 2002). The reduction in plant growth of *P. vera* and *P. atlantica* is due to a decline in cellular division and elongation (Munns, 2002). Indeed, the reduction of plant height of the seedlings is accompanied by a reduction of the number of leaves, a delay of development of the new leaves and an acceleration of the senescence of old ones. Our results are in agreement with other works (Karimi *et al.,* 2009; Chelli-Chaabouni *et al.,* 2010), and for this reason the morphological changes are adopted as alternate indicators of stress. The senescence of the old leaves contributes to preserving the soil water. This mechanism is considered a recycling program for the redistribution of resources in favor of young leaves, stems or roots (Chaves *et al.,* 2002). In *P. vera*, an acceleration of the leaf senescence at the end of the experiment was observed particularly at 80 mM of NaCl. This phenomenon was preceded by total leaf necrosis, as revealed by Chelli-Chaabouni *et al.* (2010), that constitute a sign of toxicity by accumulation and excess of salt in the leaves (Ben Ahmed *et al.,* 2008). These symptoms were often associated with a high rate of chloride (Picchioni and Graham, 2001) or of sodium in leaves (Miyamoto *et al.*, 1985; Karakas *et al.,* 2000; Chelli-Chaabouni *et al.,* 2010; Kchaou *et al.,* 2010). Under increasing saline stress, the leaf specific weight increases in *P. atlantica*, a general response of species adapted to unfavorable habitats (Poorter and Garnier, 1999). The excess of salt in soil reduced more the growth of the shoots in *P. vera* seedlings throught a significant reductions in the leaf growth and increases in the leaf senescence. However, *P. atlantica* maintains a growth less affected for the NaCl concentrations.

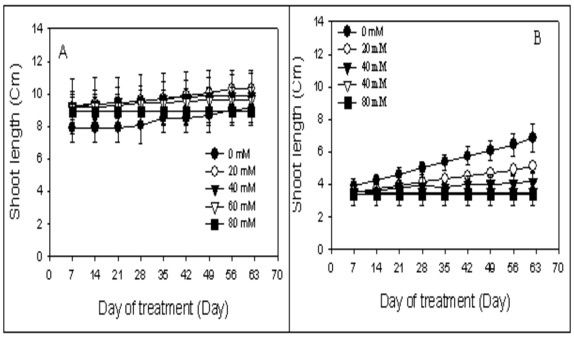

Fig. 5. Shoot length variation in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

Phytomass allocation under salt stress

Salinity is a determining element for the plant growth, particularly in arid and semi-arid areas. It induces a significant reduction of shoots growth, whereas it improves the root growth except for the highest salt concentration (80 mM). Similar effects were observed on the growth of the seedlings of pistachio tree subjected to salt stress in vivo conditions (Ranjbar *et al.,* 2002; Tavallali *et al.,* 2008; Karimi *et al.,* 2009) and in vitro (Benmahioul *et al.,* 2009). The same results were also obtained in other plants as *Olea europea* (Kchaou *et al.,* 2010). According to Zhu (2001), the reduction of the shoots growth is an adaptive mechanism necessary to the survival of the plants exposed to abiotic stress. Plants protect the shoots against invasion from the toxic ions (Na^+ and Cl^-) by its accumulation in roots. In addition, Bouraoui *et al.* (1998) showed that the passage of the cells from the division to the elongation stage is accompanied by a modification of the respiratory metabolism. The maintenance of the root elongation and the stimulation of respiration on medium salted correspond a mechanism of tolerance to salinity (Bouraoui *et al.,* 1998). Indeed, the leaf area decreased significantly in both species, which led to the development of the root, considered as a criterion for salinity tolerance (Chelli-Chaabouni *et al.,* 2010). This would allow better use of the available water (Benmahioul *et al.,* 2009), suggesting an internal settlement mechanism, thus, the osmoregulation allows the plant to adjust its water potential while promoting root elongation and water supply.

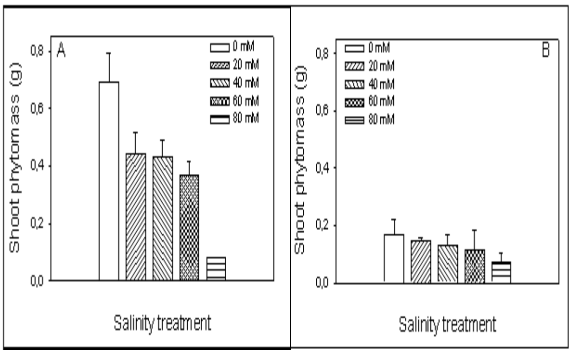

Fig. 6. Shoot phytomass in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

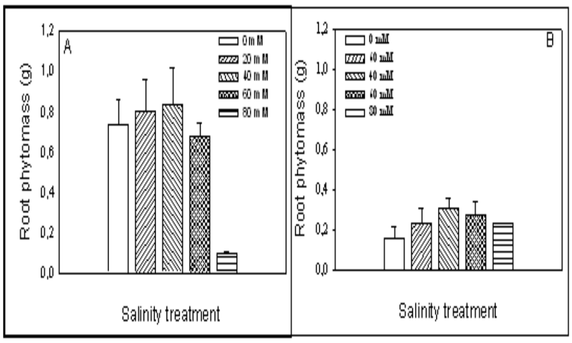

Fig. 7. Root phytomass in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

Roots are affected by abiotic stresses in many ways. Salinity induces a reduction of root system biomass through a decrease in root length and width. Cotton seedlings growing in hydroponic salt solution produced less and thinner roots with increasing salinity. Root anatomical analysis showed shorter and more nearly iso-diametrical cortical cells than those of control plants (Kurth *et al.,* 1986). Salt stress may lead to root lignifications in pistachio (Walker *et al.,* 1987). Under salinity, the results represent a preferential allowance of the resources in favor of the roots in *P. atlantica* at higher concentrations of NaCl. It maintained a higher root growth and shoots, compared to *P. vera* for the NaCl concentrations. These responses translate a tolerance of *P. atlantica* to salt stress, thus, the good performance of *P. vera* grafted on *P. atlantica* can be allotted to the adaptive ecophysiologic characteristics related particularly to root growth.

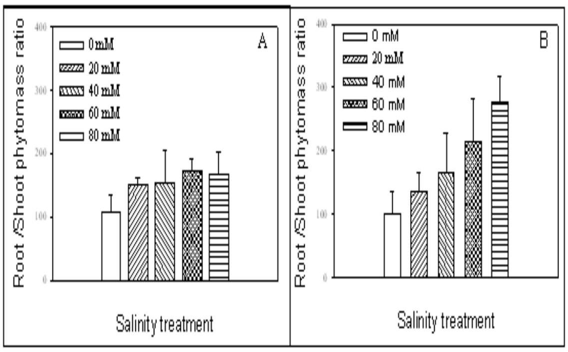

Fig. 8. Root/shoot ratio in *Pistacia vera* (A) and *Pistacia atlantica* (B) plants under salinity treatment. Each value is the average of six replicates and vertical bars represent standard errors.

Potentiality of establishment of P. vera and P. atlantica under salinity stress

Under salinity, a significant reduction in shoots was observed in both species, considered as a mechanism of tolerance necessary for the survival of plants exposed to abiotic stress (Zhu, 2001). However, *P. atlantica* maintains shoots less affected by NaCl, compared to *P. vera*. The increases in leaf specific weight and the preferential allowance of the resources in favor of the roots translate a tolerance of *P. atlantica* to salt stress, against root necrosis and a senescence of leaves in *P. vera*. This confirms that species from favourable productive habitats present high SLA, high relative growth rate (RGR) and high allocation of biomass to shoots, while species with low SLA and low RGR, allocate more biomass to roots in unfavourable habitat (Poorter and Garnier, 1999). The mechanisms explain the good performance of the seedlings of *P. atlantica*, compared with *P. vera* under higher concentration of NaCl (80 mM of NaCl). That joined the literature to explain the agricultural practice based on the use of *P. atlantica* as a rootstock of *P. vera* for a better rusticity to the abiotic constraints as salinity. The good performance of *P. vera* grafted on *P. atlantica* can be allotted to the adaptive ecophysiologic characteristics related particularly to root growth.

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